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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/666,366
Filing Date: September 19, 2003
Appellant(s)Huang et al.

Joseph T Leone
For Appellant

EXAMINER'S ANSWER

This is in response to the final office action, mailed 7/3/2008.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The following are the related appeals, interferences, and judicial proceedings known to the examiner which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal:

U.S. Application No. 10/403,395 is currently on appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct, with the exception that the grounds of the rejection as stated by appellants appears to include argument concerning the merits of the ground of rejection presented for review. It is noted that there is but a single Grounds of rejection to be reviewed on

appeal, whether Claims 1, 5, 7-10,14-18, 22,24-29,31-35,37-40 and 42-45 are unpatentable under 35 U.S.C. 103(a) over Mizutani et al. (Microbiol. Immunol., Vol 42 (8), pp 549-553, 1998) and Ambion, Inc. (TechNotes 8(2), SUPERase.In™: The Right Choice for Protecting your RNA, web page, www.ambion.com/techlibb/tn/82/823.htm, 3/27/2003.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

Mizutani et al., Microbiol. Immunol., Vol 42 (8), pp 549-553, 1998.

Ambion, Inc., TechNotes 8(2), SUPERase.In™: The Right Choice for Protecting your RNA, web page, www.ambion.com/techlibb/tn/82/823.htm, 3/27/2003.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40 and 42-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mizutani et al. (Microbiol. Immunol., Vol 42 (8), pp 549-553, 1998) and Ambion, Inc. (TechNotes 8(2), SUPERase.In^{TM}: The Right Choice for Protecting your RNA, web page, www.ambion.com/techlibb/tn/82/823.htm, 3/27/2003).

Mizutani et al., disclose a single step reverse transcription-polymerase chain reaction (RT-PCR) for the detection of Hepatitis C virus RNA. Specifically, Mizutani et al. teach a method of RT-PCR comprising heating a RNA solution to 95^{o}C for 20 minutes followed by 70 cycles of amplification (See page 550, left column third full paragraph). Mizutani et al. further note that there is a risk of contamination during manipulation as a result of the opening of the tube to add reaction mixture.

Ambion, Inc. teach that when considering where RNase contamination might originate, it becomes dear why you need to inhibit different types of RNases. Ambion, Inc teach that RNase A, for example, is a common contaminant on laboratory equipment and supplies because it is present on human skin. Ambion, Inc. teach that RNase A is used in large quantities for both plasmid and protein purification, and along with RNase T1, it is used in ribonuclease protection assays. Ambion, Inc. teach that

bacterial RNases can affect experiments that including bacterial lysates, or proteins or DNA templates that are purified from overexpression in bacteria. Ambion, Inc. teach that even commercial enzymes can be contaminated with trace amounts of RNases (all types) and environmental sources such as dust, ungloved hands, and contaminated solutions may also introduce many different types of RNase. Thus, there are many sources of RNase contamination in the laboratory.

Ambion, Inc. teach a method comprising to a first solution in which RNase contamination is a concern, adding a second solution containing an amount of an RNase inhibitor protein (i.e. SUPERase.In™) in a buffer devoid of reducing agents to yield a mixture and heating the mixture to a temperature of 67°C for 15 minutes (See figure 2 and supporting text). The RNase inhibitor SUPERase.In™ is derived from a mammalian source. Ambion further teach that SUPERase.In™ does not require DTT to function and inhibits more RNAses, at higher concentrations, under more reaction conditions than other RNase inhibitors. Ambion specifically teach that SUPERase.In™ is ideal for use in RT-PCR.

One of skill in the art at the time of filing would have been motivated to practice the methods of RT-PCR of Mizutani et al., with the addition of SUPERase.In™ as taught by Ambion, Inc. Mizutani et al. teach the reaction method as follows: It is noted that prior to the assay, the RNA being assayed for is vulnerable to the degradative action of RNases. It is at this point that one of skill in the art would be motivated to supplement the RNA containing solution or any solution that will come in contact with the RNA solution, with SUPERase.In™ ribonuclease inhibitor of Ambion, Inc., to protect the RNA

from potential RNases. The RNA solution is added to the reaction mixture containing buffer, dNTPs, primers, reverse transcriptase, and *Taq* polymerase and this reaction is heated for 1 min at 65°C, to denature the RNA, followed by 60 min at 42°C to synthesize cDNA, followed by heating to 95°C for 20 min and followed by heating to 94°c for 70 cycles. Thus the combination of the method of Mizutani et al. and Ambion, Inc. as described previously and herein, makes obvious the claimed method steps that are directed to the combination of a RNAse inhibitor devoid of a reducing agent and the addition of heat to a temperature of no less than 90°c. It is noted that the heating step of 95°C for 20 minutes is considered "a time sufficient to inhibit RNase activity present in the mixture". Additionally it is noted that the RNA present in the mixture is protected from enzymatic degradation by RNases.

It is noted to appellants that SUPERase.In ™ as taught by Ambion, Inc. is considered to be a "RNase inhibitor protein derived from rats, human placentas or recombinant human placental sources". The motivation for the inclusion of SUPERase.In™ Ribonuclease inhibitor in the methods of RT-PCR taught by Mizutani et al., is that SUPERase.In™ inhibits RNases that are known contaminants of RNA preparations. Ambion, Inc further teach that SUPERase.In™ works well in RT-PCR reactions and does not need reducing conditions or reducing agents. The reasonable expectation of success comes from the high level of skill in the art with respect to PCR amplification technologies and the results of both Mizutani et al., and Ambion, Inc.. Ambion, Inc. specifically teaches that the inclusion of SUPERase.In™ in RT-PCR reactions is ideal.

Thus the methods claimed in claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40 and 42-45 are obvious over Mizutani et al. and Ambion, Inc.

(10) Response to Argument

In response to the above rejection, Appellants submit that the rejection is improper on three grounds as bolded in appellants presented arguments.

Appellant's first reason that the rejection is improper is that appellants submit that:

I. Appellants submit that the rejection of Claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40, and 42-45 under 35 USC §103(a) over Mizutani et al. and Ambion is improper because Mizutani et al. and Ambion are silent or teach away from heating RNase inhibitors to increase RNase-inhibiting activity.

Appellants submit that the Office has not established *a prima facie* case of obviousness because Mizutani et al. are silent with respect to protecting RNA and Ambion teaches away from heating RNases. In support of Appellants position, Appellants submit that Mizutani et al. teach an RT-PCR reaction, which comprises a reverse transcription step in which an RNA solution is heated to 95°C and there is no teaching in Mizutani et al. to warrant adding an RNase inhibitor and heating it to the currently claimed temperatures. Appellants submit that from the teaching of Mizutani et al., there is no motivation to add an RNase inhibitor to the RNA-containing RT-PCR reaction taught therein and thus the motivation to add an RNase inhibitor to the RT-PCR reaction taught by Mizutani et al. must therefore derive from Ambion.

Appellants submit that Ambion teaches heating solutions containing RNase inhibitors to a temperature of 67°C and that in stark contrast, Ambion teaches that heating RNase inhibitors increases latent RNase activity. The latent RNase activity released by the RNase inhibitors upon heating would destroy any RNA in the solution. Thus, Ambion teaches that heating RNase inhibitors not only decreases ability of RNase inhibitors to inhibit degradation of RNA by RNases but stimulates RNase activity and is therefore counterproductive.

Appellants submit that against Appellants' position that Ambion's teaching that heating releases RNase activity teaches away from combining the RNase (Inhibitor) of Ambion with heating taught by Mizutani et al., the Office has stated, in relevant part:

> "[A]t the point of the obvious method at which the temperature is raised to 90°C, the RNase inhibitor is no longer necessary. Thus appellant's traversal of the motivation upon this basis is flawed" (see page 7, second full paragraph of Office Action dated July 3, 2008).

Appellants submit that in essence, the Office here has argued that the release of latent RNase activity does not detract from establishing a motivation to combine the RNase inhibitor of Ambion with the heating step of Mizutani et al. because there is no need to inhibit RNases in the heating step in Mizutani et al. in the first place. Appellants submit that if the RNase inhibitor is no longer necessary at the heating step, as the Office contends, then there is no technological motivation to combine the methods or conversely, the only justification for adding an RNase inhibitor to the heating step is that the RNase-inhibiting activity is beneficial to the RT-PCR method of Mizutani et al., in

which case, the release of latent RNase activity upon heating teaches away from the combination.

Appellant's complete argument continues to be acknowledged and not found persuasive for the reasons previously made of record and repeated herein.

It appears that appellants have taken the office's previous arguments as cited previously and above out of context of the made obvious methods which the previous office action was referring to. In order to address appellants points raised above, that Mizutani et al. are silent with respect to protecting RNA and Ambion teaches away from heating RNases, the office would like expand upon the teachings of the references of Mizutani et al. and Ambion, Inc.

Appellants are reminded that the instant rejection is based upon the combined teachings of Mizutani et al. and Ambion, Inc., not the references individually. As previously stated, one of skill in the art at the time of filing would have been motivated to practice the methods of RT-PCR of Mizutani et al., with the addition of SUPERase.In™ as taught by Ambion, Inc. With regard to appellants first point, while Mizutani et al. may be silent with regard to protecting RNA, the motivation for the inclusion of SUPERase.In™ Ribonuclease inhibitor in the methods of RT-PCR taught by Mizutani et al., is that SUPERase.In™ inhibits RNases, those proteins which are known contaminants of RNA preparations and responsible for the breakdown and degradation of RNA. Ambion, Inc. teach that when considering where RNase contamination might originate, RNases are a common contaminant on laboratory equipment and supplies

because it is present on human skin, used in large quantities for both plasmid and protein purification, as well as ribonuclease protection assays and even commercial enzymes can be contaminated with trace amounts of RNases (all types) and environmental sources such as dust, ungloved hands, and contaminated solutions may also introduce many different types of RNase. Further Ambion, Inc. teach that bacterial RNases can affect experiments that include bacterial lysates, or proteins or DNA templates that are purified from overexpression in bacteria. Thus it is clear that RNAse contamination may result from a number of different laboratory sources and one of skill in the art would know the importance of such in assaying the target of such contaminants. Further Ambion, Inc teach that SUPERase.In™ works well in RT-PCR reactions and does not need reducing conditions or reducing agents. The reasonable expectation of success comes from the high level of skill in the art with respect to PCR amplification technologies and the results of both Mizutani et al., and Ambion, Inc. Ambion, Inc. specifically teaches that the inclusion of SUPERase.In™ in RT-PCR reactions is ideal.

With regard to appellants second point above, that Ambion teaches away from heating RNases, Appellants are reminded of the method taught by Mizutani et al. which is a single step reverse transcription polymerase chain reaction with sensitivity and specificity to detect hepatitus C virus RNA in serum and liver biopsy specimens obtained from patients. Mizutani et al. teach the reaction method as follows: It is noted that prior to the assay, the RNA being assayed for is vulnerable to the degradative action of RNases. It is at this point that one of skill in the art would be

motivated to supplement the RNA containing solution or any solution that will come in contact with the RNA solution, with SUPERase.In™ ribonuclease inhibitor of Ambion, Inc., to protect the RNA. The RNA solution is added to the reaction mixture containing buffer, dNTPs, primers, reverse transcriptase, and *Taq* polymerase and this reaction is heated for 1 min at 65°C, to denature the RNA, followed by 60 min at 42°C to synthesize cDNA. At this point of the reaction method, the previously vulnerable RNA, being assayed, has now been converted to cDNA, which is no longer vulnerable to the degradative action of RNases. It is at this point of the reaction method that the action of Ambion, Inc.'s SUPERase.In™ is no longer necessary as it presumably has completed its intended protective function and the reaction mixture, including the SUPERase.In™ is then heated to 95°C, for 20 minutes, followed 55°C, 72°C and94°c for 70 cycles. At this point in the made obvious method the activity of the SUPERase.In™ ribonuclease inhibitor is no longer needed, however, it is not removed from the reaction mixture because it does not hurt or impede the subsequent reactions. Thus the combination of the method of Mizutani et al. and Ambion, Inc. as described previously and herein, makes obvious the claimed method steps that are directed to the combination of a RNAse inhibitor devoid of a reducing agent and the addition of heat to a temperature of no less than 90°C. Thus while Ambion, Inc. may teach away from heating RNases, this conclusion is specific to the context upon which such is done and this is not relevant to the method made obvious by the combination of Mizutani and Ambion, Inc. as described above.

Thus Appellants submission that the rejection of Claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40, and 42-45 under 35 USC §103(a) over Mizutani et al. and Ambion, Inc. is improper because Mizutani et al. and Ambion are silent or teach away from heating RNase inhibitors to increase RNase-inhibiting activity is not found persuasive for the reasons discussed above.

Appellants' second argued reason that the rejection is improper is that appellants submit that:

II. There is no technological reason or motivation to combine Mizutani et al. and Ambion.

In support of this position, appellants submit in Mizutani et al., the RNA-containing solution is heated to 95°C only after the HCV RNA template has been reverse transcribed, when the stability of the RNA is inconsequential to the outcome of the assay. The Office is in agreement with this point. Specifically, the Office has stated, in relevant part, "[A]t the point of the obvious [i. e., Appellants'] method at which the temperature is raised to 90°C, the RNase inhibitor is no longer necessary" (see page 7, second full paragraph of Office Action dated July 3, 2008). Appellants submit that there is no motivation or technical reason to add an RNase inhibitor to the protocol of Mizutani et al. at the point at which the reaction temperature is elevated to 95°C because it would provide no benefit to the assay. On this ground, Appellants submit that the Office has not established *a prima facie* case of obviousness. Appellants point is acknowledged

and understood, however, while it is agreed that it would provide no benefit to the assay to add the RNase inhibitor at the point at which the temperature is elevated to 95°C, such is not the case for adding the RNase inhibitor prior to the point in which the RNA has been converted to cDNA as discussed above and appellants claim does not preclude from the earlier addition of the RNase Inhibitor, followed by the conversion of RNA to cDNA and then the heating step.

As discussed above it is noted that prior to the assay, the RNA being assayed for is vulnerable to the degradative action of RNases. It is at this point that one of skill in the art would be motivated to or have technological reason to supplement the RNA containing solution or any solution that will come in contact with the RNA solution, with SUPERase.In™ ribonuclease inhibitor of Ambion, Inc., to protect the RNA. The RNA solution is added to the reaction mixture containing buffer, dNTPs, primers, reverse transcriptase, and *Taq* polymerase and this reaction is heated for 1 min at 65°C, to denature the RNA, followed by 60 min at 42°C to synthesize cDNA. At this point of the reaction method, the previously vulnerable RNA, being assayed, has now been converted to cDNA, which is no longer vulnerable to the degradative action of RNases. It is at this point of the reaction method that the action of Ambion, Inc.'s SUPERase.In™ is no longer necessary as it presumably has completed its intended protective function and the reaction mixture, including the SUPERase.In™ is then heated to 90°C for 20 min, followed by 55°C, 72°C, 94°c for 70 cycles. Appellants claims still encompass the discussed obvious method, even if at the time of heating the RNase Inhibitor is inactivated. At this point in the made obvious method the activity of the SUPERase.In™

ribonuclease inhibitor is no longer needed, however, it is not removed from the reaction mixture because it does not hurt the subsequent reactions. Thus the combination of the method of Mizutani et al. and Ambion, Inc. as described previously and herein, makes obvious the claimed method steps that are directed to the combination of an RNAse inhibitor devoid of a reducing agent and the addition of heat to a temperature of no less than 90°c.

Appellants further contend in response to the above and previously argued position of the office for the motivation to combine the references that:

1. Ambion does not teach that RNases are known contaminants of all RNA preparations; and

2. Ambion does not identify RT-PCR as an assay in which RNase contamination is inherently a concern.

Appellants submit that because neither of the cited prior art references provides any suggestion that RNases are known contaminants in RT-PCR reactions, Appellants submit that the Office's response to Appellants' position that there is no technological reason or motivation to combine the two references is insufficient to establish *a prima facie* case of obviousness.

In response, as stated above, one of skill in the art at the time of filing would have been motivated to practice the methods of RT-PCR of Mizutani et al., with the addition of SUPERase.In™ RNAse Inhibitor as taught by Ambion, Inc. The motivation for the inclusion of SUPERase.In™ Ribonuclease inhibitor in the methods of RT-PCR

taught by Mizutani et al., is that SUPERase.In™ inhibits RNases, those proteins which are known to be contaminants of RNA preparations and responsible for the breakdown and degradation of RNA. Ambion, Inc. teach that when considering where RNase contamination might originate, RNases are a common contaminant on laboratory equipment and supplies because it is present on human skin, used in large quantities for both plasmid and protein purification, as well as ribonuclease protection assays and even commercial enzymes can be contaminated with trace amounts of RNases (all types) and environmental sources such as dust, ungloved hands, and contaminated solutions may also introduce many different types of RNase. Further Ambion, Inc. teach that bacterial RNases can affect experiments that include bacterial lysates, or proteins or DNA templates that are purified from overexpression in bacteria. Thus it is clear that RNAse contamination may result from a number of different laboratory sources and one of skill in the art would know the importance of such in assaying for RNA, the target of such a contaminant. Further Ambion, Inc. teach that SUPERase.In™ works well in RT-PCR reactions and does not need reducing conditions or reducing agents. The fact that Ambion, Inc. teach that the laboratory and its equipment, and supplies including commercially prepared enzymes are a common source of RNAses, and the teaching that SUPERase.In™ RNase inhibitor is ideal for RT-PCR assays suggests that such RT-PCR assays are a potential point of concern regarding RNases as these are performed in a laboratory and potentially exposed to many of the for mentioned sources of RNase contamination and thus one of skill in the art would be motivated to include such in an assay in which “potential” degradation of RNA is a concern and in which it

has been determined to perform ideally. The reasonable expectation of success comes from the high level of skill in the art with respect to PCR amplification technologies and the results of both Mizutani et al., and Ambion, Inc. Ambion, Inc. specifically teaches that the inclusion of SUPERase.In™ in RT-PCR reactions is ideal.

Thus while RNases may not be known as inherent contaminants in RT-PCR reactions, Ambion, Inc. teach that RNases are known contaminants in the laboratory and thus one of skill in the art would be so motivated to take minor additional measure to protect ones experiment, especially if the benefit far out ways the cost.

Appellants' third argued reason that the rejection is improper is that appellants submit that:

III. The rejection is improper because Mizutani et al. and Ambion do not teach heating an RNase inhibitor for a time sufficient to inhibit RNase activity:

Appellants submission that even if the references were to be combined, they fail to teach all the required elements of the claims, because the independent claims require heating an RNase for a time sufficient to inhibit RNase activity, is not persuasive because as stated previously and above, that method that is made obvious by the combination of Mizutani et al. and Ambion, Inc. comprises the addition of SUPERase.In™ ribonuclease inhibitor to a solution containing RNA or to which RNA will be added, followed by heating to a temperature of 95°C for 20 min. The heating to 95°C for even a split second meets the limitation of the claim of heating the mixture of step (a) to a temperature no less than about 90°C for a “time sufficient to inhibit RNase activity

present in the mixture". The teachings of both Mizutani et al. and Ambion, Inc. are acknowledged and it is realized that each of the references do not teach such individually, however, it is the combination of the references that is the basis of the rejection. Thus it is believed that "heating the mixture of step (a) to a temperature no less than 90°C for a time sufficient to inhibit RNase activity present in the mixture" is met by heating for a mere split second. If it is appellant's opinion that such is not the case, perhaps appellants could clarify as to what exactly "a time sufficient to inhibit RNase activity present in the mixture" is? It is the position of the office that this "time limitation" associated with the heating step is met by the made obvious claim.

Thus claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40 and 42-45 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Mizutani et al. and Ambion, Inc.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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